7. Kennedy, F., Elsberg, C. A. and Lambert, C. I.: A peculiar undescribed disease of the nerves of the cauda equina. Am. Jour. Med. Sc., 1914, cxlvii, 645–667.
8. Laignel-Lavastine and Verliac: Syndrome de l'hémi-queno do cheval par méningoradiculite syphilitique. Rev. neurol., 1903, xvi, 179–182.
9. Müller, L. R.: Untersuchungen über die Anatomie und Pathologie des untersten Rückenmarksabschnittes. Deutsch. Ztschr. f. Nervenh., 1893, iv, 1–92.
10. Reynolds, C. E.: Obscure inflammations of the cauda equinn: Report of case and operation. Jour. Am. Med. Assn., 1919, lxxii, 23.
11. Sanders, W. E.: A case of motor aphasia and a case of cauda equinn disease with remarks on topical diagnosis. Interstate Med. Jour., 1918, xxv, 662–667.
12. Schmoll, E.: Tumor of the cauda equina. Am. Jour. Med. Sc., 1906, cxxxi, 133–143.
13. Spiller, W. G.: Tumors of the cauda equina and lower vertebræ. A report of nine cases: Seven with necropsy, three with operation. Am. Jour. Med. Sc., 1908, cxxxv, 365–403.
14. Spiller, W. G.: Congenital and acquired enuresis from spinal lesion: (a) Myelodysplasia. (b) Stretching of the cauda equina. Am. Jour. Med. Sc., 1916, cli, 469–475.
15. Steinke, C. R.: Spinal tumors: Statistics on a series of 330 collected cases. Jour. Nerv. and Ment. Dis., 1918, xlvii, 418–426.

ERYTHROPOIETIC ACTION, CUMULATIVE EFFECT AND ELIMINATION OF GERMANIUM DIOXIDE.

By John Hughes Müller, M.A., Ph.D.

and

Miriam Stewart Iszard, M.A.

(From the John Harrison Laboratory of Chemistry and the School of Public Hygiene, University of Pennsylvania, Philadelphia, Pa.)

The importance of germanium dioxide as an erythropoietic agent has recently been established by the investigations of Hammett, Nowrey and Müller. This experimental work was conducted at the Wistar Institute of Anatomy and Biology, Philadelphia, and the article treating of the results appeared in the *Journal of Experimental Medicine*, February, 1922.

The abstract of this article pointed out that the tests were conducted on albino rats. The results of experimentation showed that "there took place, as the result of the administration of the compound, a marked and statistically valid rise in the number of the erythrocytes. There was no corresponding leukemia. Autopsy of the treated rats showed, on gross inspection, that the bone-marrow and liver probably participate in the reaction which results in the erythropoiesis."[1]

As an outgrowth of the above-mentioned work the authors of this paper have conducted several series of tests, which work was

[1] Hammett, Nowrey and Müller: Erythropoietic Action of Germanium Dioxide, Jour. Exper. Med., 1922, 35.

carried out on the guinea-pig, rabbit, dog and man. The investigation was undertaken with the purpose of finding out: (*a*) The erythropoietic action of germanium dioxide on animals other than the albino rat; (*b*) the toxic effect of large doses; (*c*) the cumulative effect of the compound in the system; (*d*) the elimination of the compound from the system.

Previous Work with Germanium Dioxide. A search of the literature shows that animal experimentation with germanium dioxide is comparatively a new field of work. Müller in his article in the *Journal of the American Chemical Society*, which deals with the determination of the atomic weight of germanium, has given methods for the preparation of germanium compounds in a high state of purity.[2]

The next work with germanium was that dealing with "The Relative Toxicity of Germanium and Arsenic in the Albino Rat." This work was conducted by Hammett, Müller and Nowrey, and the publication of their findings appeared in the *Journal of Pharmacology and Experimental Therapeutics*. Primarily, this work was suggested by the close relationship existing between arsenic and germanium in their respective positions in the periodic system, in which they are adjacent in series. The solubility of germanium dioxide in water is the most striking connection between this oxide and the oxides of arsenic, in which respect germanium departs from the behavior of the other elements in Group IV of the periodic system and resembles arsenic, holding the adjacent position in Group V.

The results of the findings of the above authors showed that "germanium dioxide can be administered subcutaneously in the albino rat in doses up to 180 mg. per kilo of body weight with no harmful effects, whereas arsenic exhibits fatal results in a ratio of 8 mg. per kilo of body weight. Therefore, germanium does not possess a toxicity for the living animal organism such as exhibited by arsenic."[3]

Later came the work by the same authors dealing with the erythropoietic action of germanium dioxide, previously mentioned in this paper.

As far as the authors of the present paper can ascertain these above-mentioned articles are the only reports of work thus far conducted in connection with the physiological action of germanium.

Present Investigations. The germanium dioxide used throughout the present work was a portion of the original atomic weight material belonging to one of us.

[2] Müller, J. H.: Atomic Weight of Germanium, Jour. Am. Chem. Soc., 1921, 43, 1083.

[3] Hammett, Müller and Nowrey: Relative Toxicity of Germanium Dioxide and Arsenic for the Albino Rat, Jour. Pharmacol. and Exper. Therap. (Accepted for publication.)

The authors wish to state that the solubility of germanium dioxide in water has been redetermined by them, and it has been shown that a freshly prepared solution has a strong tendency to supersaturation and colloid formation. Such a supersaturated solution at 26° C. contains 0.006632 gm. of germanium dioxide per cc, but on long standing deposits a considerable quantity of the hydrated oxide. Solutions of this concentration were at first employed in this experimental work, but later were substituted by a stable saturated solution containing 0.004684 gm. of germanium dioxide per 1 cc, representing the true solubility of the pure oxide in water.

The laboratory animals used in all the tests were kept in individual wire cages and were held under observation for one week preceding experimental work, in order for them to become acclimated to their new conditions. During this period, as well as during the period of experimentation, the amount and character of the food were controlled, carrots, cabbage and water being given. Weight determinations were likewise made during the entire period at intervals of twenty-four hours.

All injections were made with a sterilized Luer 10 cc hypodermic syringe.

All blood counts were made in a blood-counting chamber having the Zappert-Neubauer type of ruling. The technic employed in making the blood counts was that as outlined in Stitt's *Practical Bacteriology Blood Work and Parasitology.*[4]

Erythropoietic Action of Germanium Dioxide. A preliminary test was run on a series of eight guinea-pigs to determine:

1. Whether an appreciable increase in erythrocyte count followed dosage with germanium dioxide.
2. Whether there was an accompanying increase or decrease in body weight.
3. Whether there was an increase in hemoglobin content.
4. Whether there was a difference between method of administration and increase in red blood cell count.

The germanium dioxide used in testing this series contained 6.632 mg. of germanium dioxide per 1 cc. The amount of germanium dioxide given was calculated on the basis of 100 mg. per kilo of body weight.

Of this series 3 animals received subcutaneous injections, 3 animals received intraperitoneal injections and 2 were used as controls.

The results of this preliminary series of tests showed that:

1. There was an appreciable increase in erythrocyte count, since it was possible to demonstrate, in all the animals treated, a rise of at least 1,000,000 over the normal count.

[4] Stitt, E. R.: Practical Bacteriology, Blood Work and Parasitology, sixth edition. P. Blakiston's Son Co., pp. 249–252.

TABLE I.—SHOWING THE ERYTHROPOIETIC ACTION OF GERMANIUM DIOXIDE IN A SERIES OF FOUR GUINEA-PIGS.

	Animal No. III$_1$.			Animal No. III$_2$.			Animal No. IV$_1$.			Animal No. IV$_2$.		
Days.	Dose of GeO_2 in mg.	Erythrocyte count in c.mm.	Weight of animal in gm.	Dose of GeO_2 in mg.	Erythrocyte count in c.mm.	Weight of animal in gm.	Dose of GeO_2 in mg.	Erythrocyte count in c.mm.	Weight of animal in gm.	Dose of GeO_2 in mg.	Erythrocyte count in c.mm.	Weight of animal in gm.
0	27.0350	4,500,000	371	20.4088	4,420,000	302	30.4400	5,000,000	415	20.4088	4,520,000	305
1	.....	5,200,000	352	.....	4,050,000	342	.....	5,140,000	431	.....	5,210,000	370
2	.....	5,520,000	302	.....	5,070,000	330	.....	5,320,000	427	.....	0,100,000	383
3	.....	5,120,000	300	.....	5,240,000	330	.....	5,340,000	429	.....	5,200,000	390
4	.....	4,440,000	378	.....	4,800,000	330	.....	5,100,000	450	.....	5,520,000	384
5	.....	4,570,000	300	.....	5,110,000	330	.....	5,100,000	453	.....	5,870,000	391
0	40.84	4,570,000	345	.....	4,470,000	330	.....	5,100,000	400	.....	5,720,000	371
7	.....	4,000,000	340	.....	5,340,000	330	40.84	5,100,000	403	.....	4,000,000	408
8	.....	4,030,000	340	.....	5,400,000	354	.....	0,000,000	400	.....	4,050,000	407
0	.....	5,100,000	300	40.84	4,580,000	350	.....	5,900,000	353	40.84	4,010,000	405
10	.....	5,740,000	305	.....	5,280,000	345	.....	5,090,000	340	.....	5,500,000	397
11	.....	5,070,000	375	.....	4,810,000	352	.....	0,880,000	330	.....	0,050,000	370
12	.....	5,040,000	375	.....	4,700,000	355	.....	0,430,000	325	.....	5,000,000	380
13	.....	.....	...	.....	4,570,000	357	.....	5,400,000	297	.....	5,830,000	398
14	.....	.....	...	.....	4,930,000	352	.....	.....	...	.....	0,000,000	401
15	.....	.....	...	.....	5,000,000	398	.....	.....	...	.....	4,020,000	397

2. As regards the body weight the animals receiving but one dose based on 100 mg. per kilo of body weight showed no change or a slight increase in body weight, whereas those given more than one dose on successive days showed a decrease in body weight, from which they gradually recovered after dosing was discontinued.

3. Accompanying an increase of 1,000,000 in erythrocyte count there was an increase in hemoglobin approximating a rise of 8 to 10 per cent above the normal hemoglobin reading.

4. Since all animals treated showed a rise in the erythrocyte count, which rise showed no variation between subcutaneous and intraperitoneal injections, the authors concluded that the mode of administration does not affect the erythropoietic action of the germanium dioxide.

A. Erythropoietic Action of Germanium Dioxide in Guinea-pigs. Following the preliminary series a more detailed series of tests was run on 4 guinea-pigs, observations being made at intervals of twenty-four hours as regards body weight and erythrocyte count.

In this series the dose was administered intraperitoneally.

The normal erythrocyte count and weight of the animal was taken just prior to the initial dose.

The germanium dioxide used in this series was a solution containing 4.684 mg. per 1 cc.

Table I shows the results of this experimental work:

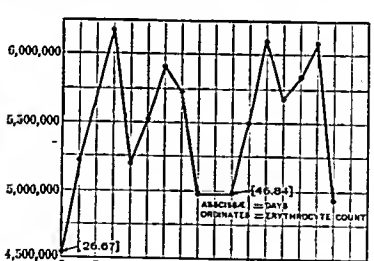

CHART 1.—Curve showing the erythropoietic action of germanium dioxide in a guinea-pig. Two doses of GeO_2 given intraperitoneally; each cc of solution = 4.68 mg. GeO^2. Arrows above indicate days on which doses were received, and bracketed numbers indicate the number of mgs. received on that particular day

Table I shows that following the administration of a dose based on 100 mg. per kilo of body weight there was (*a*) an accompanying rise in erythrocyte count, (*b*) there was a change in the body weight in the case of Nos. III_1 and III_2 there occurred a slight decrease in body weight at first followed by a rise above the weight taken just prior to the initial dose; in case of Nos. IV_1 and IV_2 a gradual

increase in body weight was seen. However in all 4 animals when the amount of dose was doubled there was noted a decrease in body weight followed by an increase in 3 of the animals, which increase was above the first weight taken.

This rise and fall in the erythrocyte count can best be shown if plotted graphically. Chart I is the erythrocyte curve of animal No. IV_2, the weights and erythrocyte counts of which are given in Table I.

B. Erythropoietic Action of Germanium Dioxide in a Rabbit. For this experiment a 2479 gm. male rabbit was used. This rabbit was given an intraperitoneal injection of germanium dioxide containing 4.684 mg. per 1 cc. In other words the rabbit received 234.2 mg., which dose based upon kilo of body weight is 94.4 mg. per kilo. Following this injection records of the weight and erythrocyte count were made for a period of two weeks at approximately twenty-four-hour intervals. Table II shows the results obtained:

TABLE II.—ERYTHROPOIETIC ACTION OF GERMANIUM DIOXIDE IN A RABBIT WHICH HAD RECEIVED 234.2 MG. OF GERMANIUM DIOXIDE IN ONE DOSE.

Days.	Weight of rabbit in grams.	Erythrocyte count in cubic millimeter.
0	2479	5,350,000
1		
2	2458	5,010,000
3	2375	0,170,000
4	2325	0,430,000
5		
6	2289	5,830,000
7	2504	0,730,000
8	2330	0,210,000
9	2215	0,890,000
10		
11	2390	7,120,000
12	2320	0,090,000
13	2329	0,440,000
14	2395	5,950,000
27	....	6,030,000

Table II shows that accompanying a single dose there is a rise and fall in the erythrocyte count which extends over a period of two weeks, reaching its maximum on the ninth day, an increase of 1,540,000 over the normal count. On the fourth and thirteenth days are seen increases of 1,000,000 over the normal count. At the end of the second week the count becomes a constant, as was proved by allowing thirteen days to elapse before the final count. This determination showed no appreciable rise.

Chart II, which is the graphic chart of erythrocyte counts of the rabbit demonstrates a similar condition following the dose of germanium dioxide to that exhibited in the guinea-pig in Chart I. The dotted line in the curve indicates the lapse of thirteen days.

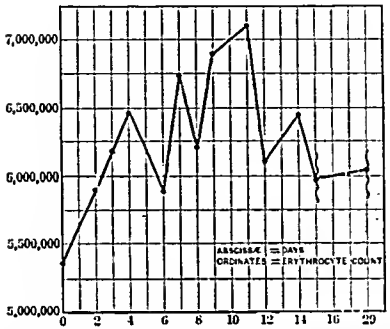

CHART II.—Curve showing erythropoietic action of germanium dioxide in a rabbit. One dose of GeO_2 given intraperitoneally on 0 day, 50 cc of n solution being given which contain 4.684 mg. of GeO_2 per cc.

C. Erythropoietic Action of Germanium Dioxide on a Dog. A St. Bernard dog, aged ten years, weighing about 62 kg., was kept under observation for forty-eight days, during which time doses of germanium dioxide were given subcutaneously in the region of the breast at stated intervals, and the erythrocyte count was made every twenty-four hours.

Table III and the accompanying graphic chart show the nature and results of this experimental work.

Before interpreting the results demonstrated in Table III and Chart III the authors wish to state that the dog gave an initial count of 2,000,000 below the average normal count, which count for a dog is 6,500,000.[5] This subnormal erythrocyte count can possibly be attributed to the age of the dog.

As the result of continued dosing in the first nine days there was seen an increase in red blood corpuscles reaching a maximum on the fourth day after the first dose was received, which increase was 3,150,000 over the initial count. On the fifth day a decrease was demonstrated, followed by a recovery on the sixth day. Accompanying this continued dosing there was seen a fluctuating rise and fall in the curve.

Following the discontinuance of the dose on the ninth day the count gradually rose, reaching on the seventeenth day, which is eight days after discontinuance of the dose, a maximum comparable to that reached on the fourth day. Without any further dosing the erythrocyte count dropped, reaching its lowest point on the twenty-first day, eleven days after the cessation of dosing.

[5] Plimmer, R. H. A.: Practical Organic and Biochemistry, Longmans, Green & Co., 1920, p. 473.

Again a rise was demonstrated, and on the twenty-sixth day, or sixteen days after the last dose, a second maximum was reached which was just 1,000,000 less than the first. Following this second rise the erythrocyte count gradually approached the normal reading, becoming more or less constant at a count about 1,000,000 above the initial count.

TABLE III.—SHOWING ERYTHROPOIETIC ACTION OF GERMANIUM DIOXIDE IN A DOG. TOTAL DOSE = 1265.7 MG.

Days.	Erythrocyte count in cubic millimeter.	Subcutaneous dose of germanium dioxide in cubic centimeters of solution.	Dose of germanium dioxide in milligrams.
0	4,550,000	17.4	115.4
1	5,390,000	15.5	102.8
2	0,000,000	15.3	101.5
3	6,700,000	15.5	102.8
4	7,700,000	15.3	101.5
5	6,390,000	15.3	101.5
0	7,320,000	15.3	101.5
7	7,200,000	15.3	101.5
8	.....	15.3	101.5
0	7,480,000	15.3	101.5
10			
11	7,210,000		
12	6,210,000		
13	6,300,000		
14	6,400,000		
15	0,500,000		
10			
17	7,800,000		
18	7,300,000		
19	0,600,000		
20	6,340,000		
21	5,140,000		
22			
23	5,450,000		
24			
25	0,590,000		
20	6,750,000		
27	5,900,000		
28	5,700,000		
29	5,790,000		
30	5,000,000		
31	5,100,000		
32	5,060,000	50.0	234.2
33	5,980,000		
34	5,660,000		
35	0,760,000		
36			
37	0,630,000		
38	0,970,000		
39			
40	0,100,000		
41	5,750,000		
42			
43	0,250,000		
44	5,000,000		
45			
46	5,660,000		
47			
48	5,650,000		
63	0,250,000		

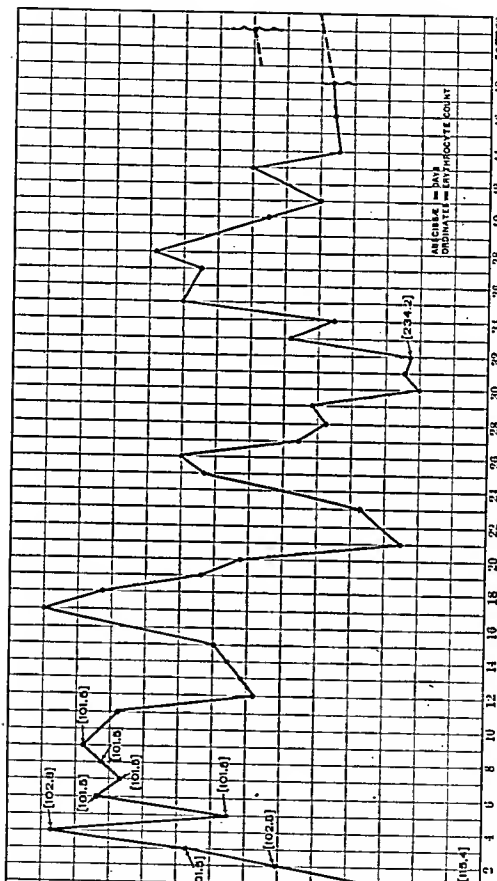

CHART III.—Chart showing the erythropoietic action of germanium dioxide in a dog. Doses of GeO_2 given subcutaneously. Arrows above indicate days on which doses were received, and bracketed numbers indicate the number of mgs. received on that particular day.

Whea the count was demoastrated as heing a constant a large dose of 234.2 mg. of germanium dioxide in solution was injected into the dog. Following this dose there can readily he seen hy referring to Curve III that a rise and fall in erythrocyte count occurred comparahle to that demonstrated in previous continued dosing.

Summing up the above results it has been demonstrated that oae large dose shows the same effect as regards increase in erythrocyte count that continued small doses on successive days produce. Also that the reaction of germaaium dioxide is rapid, requiring four days to reaeh its maximum and approximately two weeks following discontinuanee of the dosage before the erythrocyte count hecomes a constant.

By referring to Chart III and Tahle III it is seea that the count ohtained on the sixty-third day indicates a rise over that made on the forty-eighth day. However, the interesting faet is that this last count is approximately that of a aormal dog.[6]

D. Erythropoietic Action of Germanium Dioxide in a Man Aged Thirty-nine Years. In carrying out this phase of the work the dose of germanium dioxide was administered through the mouth, an aqueous solutioa of the dioxide being taken on an empty stomaeh in each instance, oae-half hour before a meal. Prior to the initial dose the weight and red blood cell count were takea.

Observations of the erythrocyte count and hody weight were made on this individual over a period of forty-two days. Table IV and Chart IV give a consideration of the work done and the results ohtained.

Both Tahle IV and Chart IV show that the rise and fall in the erythrocyte count, accompaaying and following dosage of germanium dioxide in man, is comparable with that exhibited in the case of lahoratory aaimals. At the end of the second week following the discontinuance of the dose the count became a constant, further confirmed by a count made fifteen days later. This is shown on the ehart hy a dotted line.

Ia addition the results for man show that a dose ahsorhed through the stomach produces the same effect in increasing the erythrocyte count as subcutaneous and intraperitoneal injections did in the case of the laboratory animals.

In summing up the work on the erythropoietie action of germanium dioxide the following conclusioas have been drawn.

1. Following a dose of germanium dioxide hased upoa 100 mg. per kilo of hody weight there is a marked rise in erythrocyte count.

2. Following doses of germanium dioxide the authors were able to demonstrate in each case a rise in red blood cell count of at least 1,000,000 ahove the count ohtained just preceding the first dose.

[6] Loc. cit.

The maximum erythrocyte count obtained was a rise of 3,000,000 in the case of the dog, the initial count of which was below normal.

3. Following a single dosage there follows a rise and fall in the erythrocyte curve explainable probably on the grounds of periodicity, a certain linking up of the compound, causing a stimulation of the blood-producing organs, followed by the rapid production of erythrocytes. This opinion is supported by the hyperemic condition of the bone-marrow, plainly seen on gross examination, at autopsy, of the long bones of all animals injected.

TABLE IV.—SHOWING THE ERYTHROPOIETIC ACTION OF GERMANIUM DIOXIDE IN A MAN. DOSES TAKEN THROUGH THE MOUTH. TOTAL DOSE = 2408.7 MG.

Days.	Erythrocyte count in cubic centimeter.	Dose of germanium dioxide in cubic centimeter of solution.	Dose of germanium dioxide in milligrams.	Body weight in kilos.
0	5,000,000	15.3	110.5	61.01
1	6,550,000	15.3	110.5	01.00
2	6,310,000	30.0	203.0	61.69
3	6,140,000	30.6	203.0	61.78
4	5,000,000	30.6	203.0	61.40
5	6,000,000	30.6	203.0	61.91
6	6,010,000	30.6	203.0	
7	6,220,000	30.6	203.0	61.82
8	5,580,000	30.6	203.0	61.82
9	5,300,000	....	.....	02.00
10	.....	....	.....	62.05
11	5,900,000	....	.....	62.20
12	6,270,000			
13	5,850,000	....	.....	01.91
14	5,330,000	....	.....	61.90
15	4,000,000	30.6	143.2	61.40
10	5,140,000	30.0	143.2	61.23
17	5,220,000	30.6	143.2	61.30
18	.....	....	.....	61.70
10	5,350,000	....	.....	01.75
20	5,820,000	....	.....	61.75
21	5,600,000	....	.....	62.14
22	5,220,000	....	.....	01.90
23	5,560,000	....	.....	02.10
24	5,100,000			
25	4,910,000			
20	5,010,000	50.0	234.2	01.75
27	5,650,000	26.0	121.8	01.70
28	0,510,000	....	.....	01.60
29	5,890,000	....	.....	61.69
30	.....	....	.....	01.69
31	5,430,000			
32	5,540,000			
33	.....			
34	5,190,000			
35	5,460,000	....	.....	61.68
36	.....	....	.....	01.68
37	5,200,000	....	.....	61.68
38	5,540,000	....	.....	01.70
39	.....	....	.....	61.80
40	5,460,000			
41	.....	....	.....	61.90
42	5,500,000	....	.....	02.00
57	5,550,000			

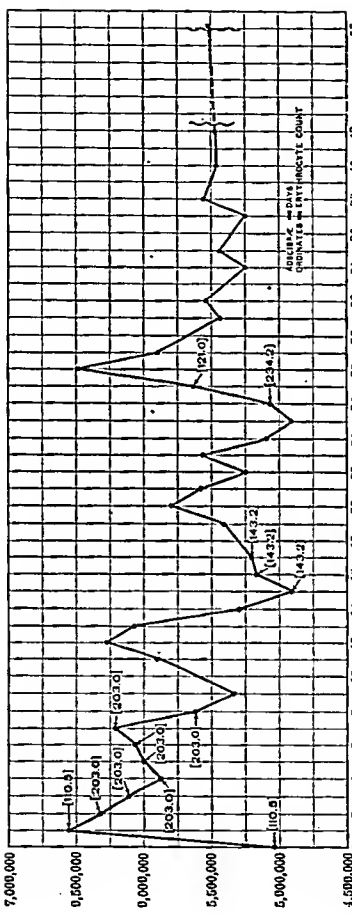

CHART IV.—Chart showing the erythropoietic action of germanium dioxide in man. Doses of GeO_2 taken through the mouth. Arrows indicate days on which doses were received, and bracketed numbers indicate the number of mgs. received on that particular day.

Toxic Action of Germanium Dioxide. Work done at the Wistar Institute of Anatomy and Biology on the toxicity of germanium dioxide in the albino rat was concerned chiefly with the administration of small doses, the largest dose given being 180 mg. per kilo of body weight which represented in actual quantity not more than 34 mg. per animal.[7]

The writers of this paper have in their work used in the various phases of this problem as much as:

Guinea-pig, 73.5 mg.
Rabbit, 234.2 mg.
Dog, 1265.7 mg.
Man, 2408.7 mg.

which doses showed no apparent toxic effect.

These amounts, however, were not given in one dose but were extended over a period of several days, with intervals elapsing. Hence the factor of elimination enters in, discussed later in this article, which work shows that germanium dioxide is rapidly eliminated from the system.

In testing out the toxic effect of germanium dioxide a series of 3 guinea-pigs was injected intraperitoneally with the germanic acid solution.

Pig A received as an initial dose 39.78 mg. of germanium dioxide.
Pig B received as an initial dose 53.04 mg. of germanium dioxide.
Pig C received as an initial dose 66.32 mg. of germanium dioxide.

The subsequent doses received by each animal were the same as the initial dose received.

Table V shows the result of administration of these quantities.

The result of this experimental work shows that the fatal dose is between 238.68 mg. and 265.20 mg. of germanium dioxide, since a pig receiving 238.68 mg. was unaffected and all receiving as much as 265.20 mg. died.

Accompanying the large doses received by these animals there was a marked decrease in body weight. At autopsy no gross pathological changes were noted except a marked hyperemic condition of the bone-marrow. Moreover, in the tests conducted to determine the erythropoietic action of germanium dioxide it was noticed that when a large dose was given there was a retardation in the increase of red blood corpuscles and an accompanying decrease in the body weight, therefore it can be concluded that germanium dioxide does have a toxic effect if taken in very large doses.

Calculating on the basis of per kilo of body weight it was found from the preceding experiment that 265 mg. of germanium dioxide proved fatal for the 3 guinea-pigs injected. Since the heaviest of these animals weighed 465 gm. we can assume that the lethal dose

[7] Hammett, Müller and Nowrey: Relative Toxicity of Germanium Dioxide and Arsenic for the Albino Rat, Jour. Pharmacol. and Exper. Therap. (Accepted for publication.)

TABLE V.—SHOWING LETHAL DOSAGE OF GERMANIUM DIOXIDE FOR A GUINEA-PIG.

Days	0		1		3		4		5		6		8		
Animal.	Body weight in gm.	Dose of GeO_2 in mg.	Body weight in gm.	Dose of GeO_2 in mg.	Body weight in gm.	Dose of GeO_2 in mg.	Body weight in gm.	Dose of GeO_2 in gm.	Body weight in mg.	Dose of GeO_2 in gm.	Body weight in gm.	Dose of GeO_2 in mg.	Body weight in gm.	Dose of GeO_2 in mg.	Result.
A	495	39.78	447	39.78	449.5	39.78	430	39.78	354	39.78	336	39.78	336	39.78	Died on ninth day after first dose.
B	422	53.04	382	53.04	355.0	53.04	345	53.04	343	53.04	...	.....	...	.....	Died on seventh day after first dose.
C	393	66.32	327	66.32	325.6	66.32	315	66.32	...	.....	...	.....	...	.....	Died on fifth day after first dose.

of germanium dioxide is 265 mg. Therefore, per kilo of body weight the fatal dose would be 586 mg. of germanium dioxide.

To substantiate this conclusion a series of 4 guinea-pigs were injected, basing the dosage on 586 mg. of germanium dioxide per kilo as fatal.

TABLE VI.—SHOWING RESULTS OF FATAL DOSING WITH GERMANIUM DIOXIDE.

Animal.	Body weight in grams.	Dose of germanium dioxide in cubic centimeters.	Dose of germanium dioxide in milligrams.	Results.
Guinea-pig W_1	322	40.6	190.17	Death within 18 hours after dose.
Guinea-pig W_2	319	40.2	188.29	Death within 18 hours after dose.
Guinea-pig X_1	380	47.8	223.89	Death within 18 hours after dose.
Guinea-pig X_2	380	47.8	223.89	Death within 18 hours after dose.

Table VI shows that following one large dose, supposedly the approximate lethal dose, all 4 guinea-pigs died within eighteen hours.

Therefore, between 500 and 600 mg. per kilo of body weight is toxic for the guinea-pig. Also this amount given in one large dose has the same effect as a series of smaller doses given on successive days See Table V. Hence the toxic action is not due to cumulative effect. In all cases in which toxic action was observed an overstimulation of the blood-producing organs was seen, for in all animals that died, both in this last series as well as in the previous series, on gross examination, the bone-marrow showed a marked hyperemic condition and was dark red and seemed to be in a state of coagulation, this condition being most marked at the joints of the long bones.

It was thought necessary by the authors to ascertain whether the injection of such a large volume of solution of slightly less than isotonic concentration might have contributed to the fatal effects produced in the preceding series of guinea-pigs, hence a solution of pure sodium chloride of the same tonicity as that of the germanic acid was injected into 2 control guinea-pigs. This volume of salt solution had no apparent affect upon the animals injected.

Cumulative Effect and Elimination of Germanium Dioxide. Since this phase of the problem requires the employment of a method whereby chemical analysis of animal tissues and excreta for germanium dioxide may be made, the authors wish to here state in detail the methods employed by them in making such chemical analyses.

Quantitative Analysis of Animal Tissues and Excreta for Germanium Dioxide. The general procedure was essentially the same as that

employed by Buchanan[8] in the analysis of inorganic mixtures bearing germanium, advantage being taken of the elaboration of this method as detailed in the later work of Dennis and Papish.[9]

This method is based upon the volatility of germanium tetrachloride from an aqueous hydrochloric acid solution in a current of chlorine.

As no examination of organic matter for germanium content has been reported, the authors of this paper found it necessary to make investigation concerning the preliminary treatment of the animal organs and excreta, so that practically all the germanium could be recovered from relatively large masses of organic matter.

The following procedure was finally adopted as most satisfactory:

In the case of urine and fecal material it was necessary to treat the collected samples with an excess of sodium hydroxide to fix the germanic acid content before evaporation and ignition. Approximately 10 gm. of caustic soda were added for each 300 cc sample of urine; in the case of the fecal material this was thoroughly moistened with a concentrated solution of sodium hydroxide immediately following the collection of the sample. Such materials cannot otherwise be evaporated to dryness and ignited without loss of germanium on account of the large amounts of chlorides always present.

The samples were evaporated to dryness under a hood and the dried residues were then heated in covered porcelain dishes to slightly above the charring temperature, care being exercised to avoid overheating the bottom of the dishes, and no attempt was made to burn off the residual carbon. At a low red heat in the well-covered dish the unburned porous carbon prevents the alkali from melting down and attacking the porcelain container. In the case of the urine residues especially a complete charring is desirable, as the less strongly heated residues cause much trouble by foaming when subsequently treated with hydrochloric acid.

The charred alkaline masses were next disintegrated in a little distilled water and neutralized with hydrochloric acid, and the whole rinsed into the distilling apparatus with a large excess of 1 to 1 hydrochloric acid. Usually the volume of the liquid subjected to distillation measured from 250 to 300 cc, and about two-thirds of this volume were driven over as distillate before interruption of the distilling operation. Further small portions of distillate were collected and examined for germanium dioxide as a check upon the complete removal of the tetrachloride.

Adequate condensing surface, thorough cooling of the water in the receiver, together with a safety water-trap to prevent loss of germanium chloride in the chlorine stream, were especially necessary

[8] Buchanan: Jour. Indus. and Engin. Chem., 1916, 8, 535; 1917, 9, 661.
[9] Jour. Am. Chem. Soc., 1921, 43, 2131.

ia the estimatioa of small quantities of germanium in the large masses of organic matter present ia these determinations.

In case of the animal organs, muscle and bone samples all were digested for twenty-four hours with coacentrated hydrochloric acid in stoppered flasks (100 cc of concentrated hydrochloric acid for each 20 gm. of sample). After the addition of an equal volume of water the turbid mass was transferred to the distilling apparatus together with sufficient 1 to 1 hydrochloric acid to make the volume up to 250 cc chloriae slowly led through the system in the cold was then allowed to destroy most of the organic matter before the final distillation.

From this point the analysis of the samples of urine, fecal material and aaimal tissues were identical. The acid distillates in all these aaalyses contained not only dilute hydrochloric acid, chlorine and germanium chloride, but also appreciable quantities of chlorinated fatty esters resulting from the incomplete destruction of the organic matter by either the wet or dry methods. This was evidenced by the presence of minute oily particles or a small floceulent precipitate in the distillate.

The distillates were not filtered but treated directly with hydrogen sulphide under pressure to saturation in the cold. The precipitate, coataining germanium sulphide, pure sulphur and chlorinated fatty compounds, was then removed by filtration and digested in alcohol acidified with hydrochloric acid and saturated with hydrogen sulphide. The germanium sulphide which remained insoluble was again filtered out and washed with pure alcohol contaiaing hydrogen sulphide. The sulphide was aow converted in the usual manner to dioxide, in which condition the germanium was weighed.

By the above method germanium dioxide can be detected in as small a quantity as 0.0002 gm.

It should be noted that the concentration of the hydrochloric acid used in these analyses is much greater than that recommended n the article by Dennis and Papish[10] on the separation and determination of germanium, but the special attention to acid concentration during distillation is only necessary in the presence of arseaic, which was not present in the material here analyzed.

Ia case of the presence of arsenic in animal tissues or excreta there are two methods which may be adopted for the determination of germaaium:

METHOD I. The method given by Dennis and Papish.[11] A modified distillation method depending upon the maintenance of the arsenic in the pentavalent state in an atmosphere of chlorine, under which condition the arseaic remains non-volatile.

METHOD II. A separation of arsenic and germanium through the

[10] Loc. cit.
[11] Loc. cit.

difference in the behavior of their sulphides toward hydrofluoric acid.[12]

This latter method, in the opinion of the writers of this article, has decided advantage over Method I in the simplicity of manipulation.

Cumulative Effect of Germanium Dioxide. In order to determine whether there was any accumulation and localization of germanium dioxide in the various organs of the guinea-pig a 422-gm. animal was given 40 cc of germanium dioxide in 10 cc doses over a period of four successive days. Since each cubic centimeter of such a solution contained 6.632 mg. of germanium dioxide the animal received as a total dosage 264.8 mg.

On the fifth day the animal died and was posted, the lungs, liver, kidney, muscle and leg bones being removed and examined separately by the chemical procedure as outlined by the authors under the quantitative determination of germanium dioxide in the animal tissues.

Table VII shows the result of such analyses:

TABLE VII.—SHOWING THE RECOVERY OF GERMANIUM DIOXIDE FROM THE ORGANS OF A GUINEA-PIG WHICH HAD RECEIVED AN APPROXIMATE LETHAL DOSE OF THE COMPOUND. TOTAL DOSE OF GERMANIUM DIOXIDE RECEIVED, 264.8 MG.

Organs analyzed.	Amount of germanium dioxide recovered in milligrams.	Percentage of total dosage received.
Lung	0.9	0.34
Liver	1.4	0.53
Kidney	1.1	0.41
Muscle	0.5	0.19
Bone (legs)	0.3	0.11
Total recovery	4.2	1.58

From Table VII it is evident that the accumulation of germanium dioxide in the organs examined is small and the amount recovered due to the blood content of the organs at the time of analysis.

To confirm this statement another guinea-pig was injected intraperitoneally with a slightly smaller dose than the guinea-pig listed in Table VII. The injections in this second pig were given in the same way as the injection received by the guinea-pig of Table VII. On the fifth day following the first dose the animal was bled from the jugular vein while under an anesthetic. Nineteen grams of blood were obtained, which amount was approximately 30 per cent of the total blood content of the animal. Chemical analysis

[12] Müller, J. H.: Separation of Germanium and Arsenic, Jour. Am. Chem. Soc., 1921, 43.

for germanium dioxide was made of the blood and organs of this guinea-pig.

The results of this work appear in Table VIII:

TABLE VIII.—SHOWING RECOVERY OF GERMANIUM DIOXIDE FROM BLOOD AND ORGANS OF GUINEA-PIG WHICH HAD RECEIVED SLIGHTLY LESS THAN LETHAL DOSE. TOTAL DOSE OF GERMANIUM DIOXIDE RECEIVED, 187.3 mg.

Analyses made.	Amount recovered of germanium dioxide in milligrams.	Percentage of total dosage received.
Blood 19 gms. collected	2.8	1.49
Lung	0.5	0.26
Liver	0.5	0.26
Kidney	0.7	0.37
Bone (all long bones being analyzed)	1.0	0.53
Total amount recovered	5.5	2.91

As 2.8 mg. of germanium dioxide were recovered from 19 gm. of blood, which is about 30 per cent of the total blood content of an animal weighing 550 gm., the weight of the guinea-pig just prior to bleeding, it might be assumed that there existed in the total blood stream 14.7 mg. Moreover, it is noticeable by comparing Table VII and Table VIII that the removal of part of the blood gave a lower germanium content for the organs.

Hence, it may be assumed that the cumulative effect of germanium dioxide is negligible and that the blood stream carries as much as 0.014 per cent of germanium dioxide, this amounting to 10.49 per cent of the total dose in the entire blood stream.

Elimination of Germanium Dioxide from the System. A study of the elimination of the germanium dioxide was made upon a male rabbit weighing 2300 gm. During this period the animal was kept in a rubber-lined nutrition cage (precaution being taken to render the condition such that the samples would not come in contact with any metal). This rabbit received one large dose of germanium dioxide (234.2 mg.) intraperitoneally given in 50 cc of aqueous solution, following which dose a careful study was made of material eliminated. During the observation period of thirteen days the total urine and fecal material were collected at intervals indicated in Table IX.

Chemical analysis was made of these samples by the method as outlined previously in this article. The results of such analysis are tabulated in Table IX.

The preceding results show:

(*a*) The overdose of germanium dioxide is rapidly eliminated from the system, amounting to 139.7 mg., which is about 59.6 per cent of the total dose received. This occurred within a period of twenty-four hours following the dose, and analysis showed it to be practically entirely eliminated through the kidneys.

TABLE IX.—SHOWING ELIMINATION OF GERMANIUM DIOXIDE FROM THE SYSTEM OF A RABBIT. INTRAPERITONEAL INJECTION OF GERMANIUM DIOXIDE GIVEN DECEMBER 13, 1921. DOSAGE, 234.2 MG (50 CC OF SOLUTION).

Date of collection of samples.	Analysis of urine samples for germanium dioxide. Amount recovered in milligrams.	Percentage of original dosage.	Analysis of fecal material for germanium dioxide. Amount recovered in milligrams.	Percentage of original dosage
Dec. 14, 1921	132.7	56.66	7.0	2.98
Dec. 15, 1921	2.7	1.15		
Dec. 16, 1921	3.7	1.58	26.2	11.19
Dec. 17, 1921	2.1	0.90		
Dec. 18, 1921 Dec. 19, 1921	2.4	1.03	5.0	2.13
Dec. 20, 1921 Dec. 21, 1921	1.9	0.81	0.4	2.73
Dec. 22, 1921 Dec. 23, 1921 Dec. 24, 1921 Dec. 25, 1921	1.2	0.51		
Dec. 26, 1921 Dec. 27, 1921	0.3	0.13		
Total elimination	147.0 mg.	62.77	44.0 mg.	19.03

(*b*) After the expulsion of the overdose the elimination becomes fairly constant over a long period and the amount eliminated from the intestinal tract increases slightly.

(*c*) From the results obtained about 81.8 per cent of the total dosage was accounted for within two weeks' time. This result in a measure explains the reason for the return of the erythrocyte curve to a constant in two weeks following dosage, which fact was pointed out previously in this paper.

A study was also made to determine the elimination of germanium dioxide from the system of man through the agency of the kidneys, analysis of the urine being made at definite intervals following a dose of 35.6 mg. germanium dioxide taken in 76 cc of aqueous solution through the mouth.

Table X shows the result of such a study:

TABLE X.—SHOWING ELIMINATION OF GERMANIUM DIOXIDE FROM THE SYSTEM OF A HUMAN BEING. DOSE TAKEN INTERNALLY (SOLUTION SWALLOWED) DECEMBER 11, 1921, DOSAGE 356 MG. (76 CC OF SOLUTION).

Date of collection of samples.	Volume of urine collected. cc.	Analysis of urine sample for recovery of germanium dioxide. Amount recovered in milligrams.	Percentage of original dosage
Dec. 11, 10 A.M. to Dec. 14, 10 A.M.	2140	207.0	58.14
Dec. 14, 10 A.M. to Dec. 16, 10 A.M.	1780	0.5	1.82
Dec. 16, 10 A.M. to Dec. 17, 10 A.M.	750	2.3	0.64
Total elimination	4670	215.8	60.60

The results of this study show (*a*) that a dose taken through the mouth is absorbed into the system giving the same type of elimination as a dose given intraperitoneally into the system of the rabbit, and (*b*) the expulsion of the overdose is almost identical with that exhibited in the case of the rabbit.

Conclusions. The authors of this paper as a result of the present investigations conclude that:

1. Germanium dioxide has a decided erythropoietic action in the guinea-pig, rabbit, dog and man.
2. This erythropoietic action exerts a certain periodicity as shown in all of the erythrocyte curves obtained.
3. Relatively large doses of germanium dioxide are distinctly toxic, and from the results obtained it can be roughly calculated that the lethal dose is about 586 mg. of germanium dioxide per kilo of body weight.
4. This toxic action is not explainable on the basis of the accumulation of the compound in the system but is possibly due to an overstimulation of the blood-forming organs.
5. The quantitative method as devised by the authors for the determination of germanium dioxide in animal tissues and excreta, both in the presence and absence of arsenic, is an accurate method.
6. Germanium dioxide does not accumulate but is eliminated through the kidneys and alimentary tract. The overdose being rapidly eliminated and occurring chiefly through the agency of the kidneys, elimination by way of the alimentary tract is small.

The authors wish to express their appreciation of the kindnesses extended to them by Drs. A. C. Abbott, D. H. Bergey and H. F. Smyth during the course of this work, and to thank them also for the many valuable suggestions received.

LATENT NEUROSYPHILIS IN EIGHT PER CENT OF MEDICAL PATIENTS IGNORED OWING TO NEGLECT OF LUMBAR PUNCTURE.

By H. Gray, M.D.,
BOSTON, MASS.

How impossible it is for any group of specialists to "annex syphilology as a perquisite" has recently been remarked by a specialist in this field—Stokes, in 1920. An internist may therefore be permitted to oppose those who even today talk of "syphilomania" (Gandolfo in 1919) and "Wassermania" (De Meritt in 1920). The latter, for example, expresses the views of not a few "practical men" when he says, "I use this rather slangy term, the best I can invent, for a new and hitherto unamed psychosis. . . . the